4 Packaging and the Microbial Shelf Life of Food

Dong Sun Lee
Department of Food Science and Biotechnology
Kyungnam University
Masan, South Korea

CONTENTS

4.1 INTRODUCTION

Packaging has been a key element to preserve the quality of foods in microbiological terms. Thermal preservation became possible with the availability of retortable packaging (initially champagne bottles, then metal containers, and now multilayer plastic pouches). Aseptic packaging relies on isolating the sterilized food inside barrier packaging that has been decontaminated of microorganisms. Dry food products are protected from microbial spoilage by the barrier properties of the package, which prevent moisture transfer into the food. The shelf life of microbiologically perishable foods depends greatly on packaging variables such as gas and water vapor barrier properties, atmosphere modification, and active packaging. These variables affect the microbial flora in the food, the spoilage rate due to organisms of concern, and the time for the food to become microbiologically unacceptable. This chapter discusses the shelf life characteristics of perishable foods in relation to the packaging variables, with an emphasis on the effects of package barrier properties and modified atmosphere packaging (MAP) on microbial shelf life.

Most perishable foods are vulnerable to microbial spoilage even under chilled conditions. Their shelf life is thus, for the most part, terminated when they become unacceptable due to the growth of undesirable microorganisms. Sometimes the growth of certain microbial species may even endanger consumer safety, and therefore the potential proliferation should be avoided or strictly controlled. In addition, certain pathogenic microbes such as *Salmonella* and *Campylobacter* should be totally absent from foods that are microbiologically perishable in nature. Good manufacturing practice is assumed to prevent the contamination of food by these organisms. On the other hand, for some pathogenic bacteria such as *Bacillus cereus* and *Staphylococcus aureus*, the usual practice in food processing and distribution is to reduce the level of contamination, eliminate the potential risk of their

growth to harmful levels, and thus avoid subsequent food poisoning. Alongside pathogenic organisms, certain genera of spoilage organisms dominate the microbial flora of foods, and their growth reduces sensory quality, thus limiting shelf life. As the growth of pathogenic organisms should be prevented or controlled below certain critical limits to ensure food safety, so spoilage organisms are also controlled to meet consumers' quality expectations of the food products. In the study of the microbial shelf life of food, it is generally assumed that microbial contamination and growth are controlled to avoid any health hazard. The shelf life of perishable foods is determined by the acceptable growth limit of spoilage organisms or the probability of a safe level of tolerable pathogens.

Microbial spoilage is dependent on the packaging conditions, the effect of which can be either direct or indirect. For example, high concentrations of CO_2 may directly inhibit the growth of certain microbial species, and a package that is highly permeable to water vapor may result in an increase in the moisture content of nonsterile dry food, providing an internal environment favorable for microbial spoilage. Therefore, the impact of packaging variables on the microbial shelf life of food can be understood on the basis of knowledge of the relationship between microbial spoilage and intrinsic and extrinsic factors. This chapter systematically addresses this topic by introducing and analyzing food–package–environment interactions, microbial growth kinetics, and literature data.

4.2 DEFINITION OF PARAMETERS AND TERMS FOR MICROBIAL SHELF LIFE

As with all shelf life studies, the starting point is the selection of the proper quality indices, with the index of most concern being the primary quality index. The microbial shelf life determination of food is also undertaken by identifying a fraction of the total microflora often called the *specific spoilage organisms* (SSOs) (Dalgaard et al., 2002; Koutsoumanis and Nychas, 2000). Among all the microflora, the SSOs are responsible for spoilage under a particular range of environmental conditions. The shelf life is terminated when a certain level of deterioration is reached because of the SSOs, the microbial metabolic product, or both (Mataragas et al., 2006). *Pseudomonas* spp., *Photobacterium phosphoreum*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, or *Aeromonas* spp. have been recognized as the main SSOs in fish stored under chilled conditions (Dalgaard, 1995; Taoukis et al., 1999). Lactic acid bacteria were reported to be the SSOs for vacuum-packed cooked cured meat products (Mataragas et al., 2006). Molds and yeasts were found to be the predominant microorganisms growing in natural, unpasteurized orange juices (Andres et al., 2001). Yeast growth was suspected to be the main cause of spoilage for cold-filled ready-to-drink beverages (Battey et al., 2002). Microbial spoilage of fruit products is known to be caused mostly by molds such as *Penicillium italicum* and *Penicillium digitatum* (Dantigny et al., 2005). Aerobic bacterial count has been widely used as an index for determining the microbial shelf life of many prepared foods, including meat, fish, vegetables, and cooked dishes (Buys et al., 2000; Corbo et al., 2006; Lee et al., 2008b; Vankerschaver et al., 1996). After the SSO and the range of environmental conditions under which a particular SSO is responsible for spoilage have been identified, the next step in microbial shelf life determination is to decide the population level of the SSO at which spoilage occurs and thus shelf life ends with loss of acceptability (Dalgaard, 1995; Koutsoumanis and Nychas, 2000). This step requires an understanding of the progress of microbial growth as a function of time.

Microbial growth in perishable foods can typically be represented as a function of time by the pattern shown in Figure 4.1. The growth curves are usually divided into lag, exponential, and stationary phases. This kind of segmentation of microbial growth curves is a well-established concept and can be explained by the dynamics of microorganisms in food or culture media (McMeekin et al., 1993). Storage and packaging conditions favorable for microbial spoilage result in shorter lag times and faster growth rates during the exponential phase. The cell density of the stationary growth phase may depend on the conditions: it often increases with favorable growth conditions such as increased ambient temperature but sometimes does not change with the storage conditions. Figure 4.1 presents a typical bacterial growth curve, but mold and yeast counts follow a similar

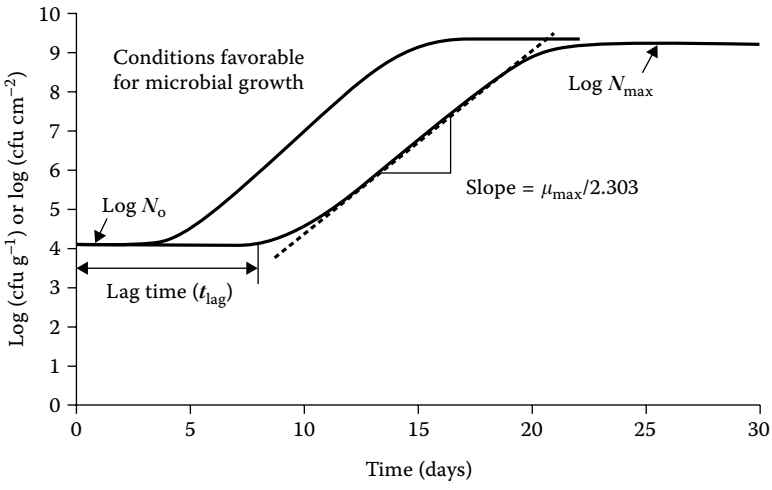

FIGURE 4.1 Typical pattern of bacterial growth on perishable food stored under constant environmental conditions.

pattern. Mold growth in radial diameter or germination percentage increase also follows a shape similar to that of Figure 4.1 (Dantigny et al., 2005).

The acceptable limit of microbial growth that determines the shelf life differs with food type, storage conditions, and defined shelf life. SSO counts of 10^5–10^8 organisms g^{-1} or cm^{-2} are commonly used as a convenient upper limit of quality and are located mostly on the linear exponential phase in Figure 4.1. For pathogenic bacteria such as *Bacillus cereus* and *Staphylococcus aureus*, 10^5 organisms g^{-1} have been used as a limit for risk management of the food supply system for prepared foods (Bahk et al., 2007; Nauta et al., 2003; Rho and Schaffner, 2007). However, the time to reach the limit based on pathogen growth should be understood as the minimum requirement for shelf life control and there should be a safety margin to give a shorter actual shelf life, this time greatly depending on the initial contamination level. Hygienic control of food preparation and processing is required so that shelf life is determined by growth of spoilage organisms rather than pathogens.

Sometimes the shelf life of foods sensitive to microbial proliferation is taken as the lag time. The start of microbial growth is often presumed to be a signal for changes in the hygienic and sensory status of the food, and thus may be taken as a conservative estimate of shelf life. The onset of an increase in bacterial count was used as a criterion for the end of the shelf life of cook-chilled or *sous vide* processed food products (Kim et al., 2002; Simpson et al., 1994). The lag time of mold or yeast growth has been used as the shelf life estimate for a prepared side dish (Lee et al., 2009).

Whether lag time or time to a level of exponential growth of the SSO is used as an estimate of shelf life, a clear and systematic determination of shelf life can be aided by describing the change in microbial population using mathematical functions (often called *primary models*). One of the most frequently used mathematical functions to describe the evolution of microbial density with time is Equation 4.1 (shown graphically in Figure 4.1), proposed by Baranyi and Roberts (1994):

$$\log N = \log N_o + \frac{\mu_{max}}{\ln(10)} \cdot A - \frac{1}{\ln(10)} \cdot \ln\left(1 + \frac{e^{\mu_{max} A} - 1}{10^{(\log N_{max} - \log N_o)}}\right) \tag{4.1}$$

where A is defined as

$$A = t + \frac{1}{\mu_{max}} \cdot \ln\left(\frac{e^{-\mu_{max} t} + 1/(e^{t_{lag}\mu_{max}} - 1)}{1 + 1/(e^{t_{lag}\mu_{max}} - 1)}\right)$$

N is the microbial count [number of organisms, usually measured in colony-forming units (cfu) g^{-1} or cm^{-2}] at time t (day), N_o is the initial density of the microbial cells (cfu g^{-1} or cfu cm^{-2}), μ_{max} is the maximum specific growth rate [inverse of the time required for the cell density to increase e (2.718)-fold, day^{-1}], t_{lag} is lag time (day), and N_{max} is the maximum cell density (cfu g^{-1} or cfu cm^{-2}).

The microbial growth model presented as Equation 4.1 can be rearranged as a differential equation to describe the instantaneous growth rate:

$$\frac{dN}{dt} = \mu_{max}\left(\frac{q}{1+q}\right)\left(1-\frac{N}{N_{max}}\right)N \tag{4.2}$$

where another state variable, q (the physiological state of the cell population), is introduced to represent the normalized concentration of an unknown substance critically needed for cell growth, whose accumulation is exponential with a specific rate of μ_{max} ($dq/dt = \mu_{max}q$).

The four parameters log N_o, t_{lag}, μ_{max}, and log N_{max} describe the progress of microbial growth over time under certain conditions. When the microbial growth pattern in Figure 4.1 is described by Equation 4.1, the parameter log N_o is presumed to be determined by the initial contamination level of the food, which is dictated by raw materials and food manufacturing conditions, whereas log N_{max} represents the maximum cell density attainable under given conditions and is usually beyond the acceptable limit of quality. Lag time (t_{lag}) and maximum specific growth rate (μ_{max}), depending on environmental conditions, directly affect the time taken to reach a certain critical level of microbial density corresponding to acceptable quality. Therefore, in dealing with the effect of packaging conditions on microbial shelf life, these two parameters are most often employed for the analysis and examined for comparative purposes. Even though the growth curve described by Equation 4.1 has curvilinear portions at the beginning and end of the exponential growth phase, the maximum specific growth rate, μ_{max}, can be assumed to represent the main part of the exponential growth. With this simplified treatment, the time (t_s) to reach a critical limit cell density of N_c, located on the exponential growth phase as the shelf life estimate, can be calculated as

$$t_s = t_{lag} + \frac{1}{\mu_{max}}\ln\left(\frac{N_c}{N_o}\right) \tag{4.3}$$

This chapter will frequently use Equation 4.3 to estimate the microbial shelf life from the kinetic parameters of microbial spoilage found in the literature.

There are other widely used primary models, such as the Gompertz and logistic functions, from which lag time and maximum specific growth rate can be similarly obtained and adopted for shelf life analysis (McKellar and Lu, 2004; McMeekin et al., 1993). As this chapter deals with the effect of packaging on the microbial shelf life of food, it will examine quantitatively the microbial growth parameters t_{lag} and μ_{max} as functions of packaging variables, leading to shelf life evaluation and analysis. More intensive treatment using complex mathematical models of the growth curve is sometimes adopted in the discipline of predictive microbiology for accurate description of microbial spoilage phenomena and for handling dynamic environmental conditions; this is beyond the scope of this chapter, and interested readers should consult McMeekin et al. (2002), Van Impe et al. (2005), and Peleg (2006).

4.3 INTRINSIC AND EXTRINSIC FACTORS AFFECTING MICROBIAL GROWTH

The growth of SSOs in packaged foods is affected by intrinsic factors (food properties) and extrinsic factors (environmental conditions inside and outside the package) (Huis is't Veld, 1996). Intrinsic

factors include pH, water activity (a_w), structure, initial contamination as a result of processing conditions, and food composition such as the presence of antimicrobials. Extrinsic factors include temperature, gaseous atmosphere, relative humidity (RH), and lighting conditions.

With developments in mathematical modeling of microbial growth, the effects of intrinsic and extrinsic factors on the primary model parameters such as lag time and maximum specific growth rate have been formulated for several spoilage organisms in microbial media or typical foods. Those models are combined and sometimes captured as computer software to predict SSO growth under certain combinations of intrinsic and extrinsic factors. Examples of such software packages are ComBase Predictor® and Seafood Spoilage & Safety Predictor®. Currently, spoilage organisms covered in their growth models include *Br. thermosphacta*, *Pseudomonas* spp., *Ph. phosphoreum*, and *Sh. putrefaciens*.

4.3.1 Intrinsic Factors

The intrinsic properties of foods vary greatly with food type, food formulation, heat treatment, and hygienic status of the processing environment. Water activity and pH are determined mostly by food type and are the main influential domain variables that allow specific microorganisms to grow or proliferate on the food. Generally, microbial growth is reduced with lower a_w, but it has been reported that a high a_w (close to 1.0) sometimes reduces slightly the growth of certain organisms (Braun and Sutherland, 2003; McMeekin et al., 1993). Most foods have a pH in the range of 3–7, and a lower pH in the acidic range usually retards microbial growth. At some lower limits of a_w and pH, microbial growth eventually stops. Figure 4.2 shows the growth rate of a cocktail of fish spoilage bacteria as a function of a_w and pH. Table 4.1 presents the approximate lower limits of a_w and pH for some food poisoning and spoilage microorganisms. Lower pH or a_w favors the growth of yeasts and molds compared with bacteria (Gould, 1996; Huis is't Veld, 1996).

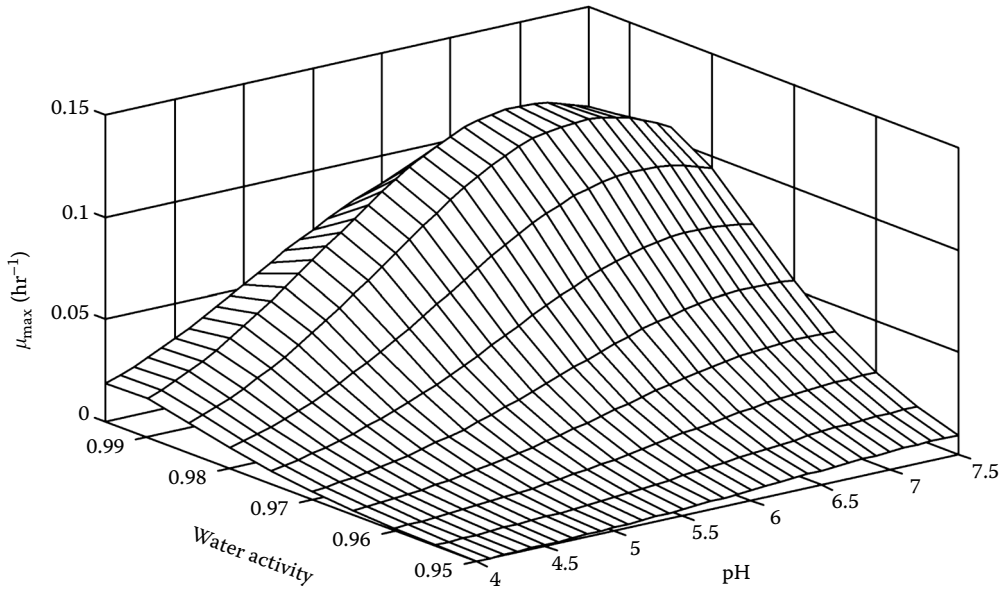

FIGURE 4.2 Maximum specific growth rate (μ_{max}) of a cocktail of *Pseudomonas* spp., *Shewanella putrefaciens*, and *Acinetobacter* spp. as a function of pH and water activity (a_w) at 5°C. (Drawn from a functional relationship reported by Braun P., Sutherland J.P. 2003. Predictive modelling of growth and enzyme production and activity by a cocktail of *Pseudomonas* spp., *Sh. putrefaciens* and *Acinetobacter* sp. *International Journal of Food Microbiology* 86: 271–282.)

TABLE 4.1
Approximate Lowest Limits[a] of Water Activity, pH, and Temperature for Growth of Some Microorganisms

Organism	Lowest Water Activity Limit	Lowest pH Limit	Lowest Temperature Limit (°C)
Bacteria			
Bacillus cereus (mesophilic)	0.93	4.9	10
Bacillus cereus (psychrotrophic)	0.93	4.9	5
Brochothrix thermosphacta	0.94	4.6	0
Campylobacter spp.	0.98	4.9	30
Clostridium botulinum (nonproteolytic)	0.97	5.0	3.3
Clostridium botulinum (proteolytic)	0.94	4.6	10
Clostridium perfringens	0.96	4.5	5
Escherichia coli	0.95	4.4	7
Lactobacillus spp.	0.93	3.0	4
Listeria monocytogenes	0.92	4.3	0
Most lactic acid bacteria	0.95	3.5	5
Pseudomonas spp.	0.97	5.0	–2
Salmonella spp.	0.95	4.0	5
Staphylococcus aureus	0.86	4.0	7
Molds			
Aspergillus flavus	0.78	2.0	3
Most molds	0.80	1.5	<0
Yeasts			
Most yeasts	0.87	1.5	–5
Saccharomyces cerevisiae	0.90	2.3	0

Source: Adapted from Leistner L., Gould G.W. 2002. *Hurdle Technologies*. New York: Kluwer Academic/Plenum Publishers, pp. 1–15; Rahman M.S., Labuza T.P. 1999. Water activity and food preservation. In: *Handbook of Food Preservation*, Rahman M. S. (Ed). New York: Marcel Dekker, pp. 339–382; Rahman M.S. 1999. pH in food preservation. In: *Handbook of Food Preservation*, Rahman M. S. (Ed). New York: Marcel Dekker. pp. 383–396; Shapton D.A., Shapton N.F. 1994. *Principles and Practices for the Safe Processing of Foods*. Oxford, UK: Butterworth-Heinemann, pp. 221–253; and other sources.

[a] Values may vary with food type and microbial strain.

Mild heat processing, such as pasteurization, inactivates vegetative microorganisms and excludes them as the cause of potential spoilage, thus limiting the spoilage microbial flora to spore formers (Gould, 1996; Huis is't Veld, 1996). The presence of preservatives may narrow down the potential list of SSOs in the food; for example, sorbic acid and its salts inhibit the growth of molds and yeasts. Figure 4.3 shows the effect of a preservative (potassium sorbate) on mold growth compared with that of a_w. The spoilage domain of intrinsic factors where SSOs are responsible for the spoilage should be examined specifically for each food item. Combined control of intrinsic factors can be utilized to preserve food safely and ensure good quality; this combination allows the food to have an adequate shelf life by incorporating low levels of additives and applying mild processes of drying and heating. Such a technique is often called *hurdle technology* (Leistner and Gould, 2002).

4.3.2 Extrinsic Factors

Storage temperature is the most influential environmental factor affecting microbial spoilage of foods. All microorganisms have an optimal temperature at which their growth is maximal. Above

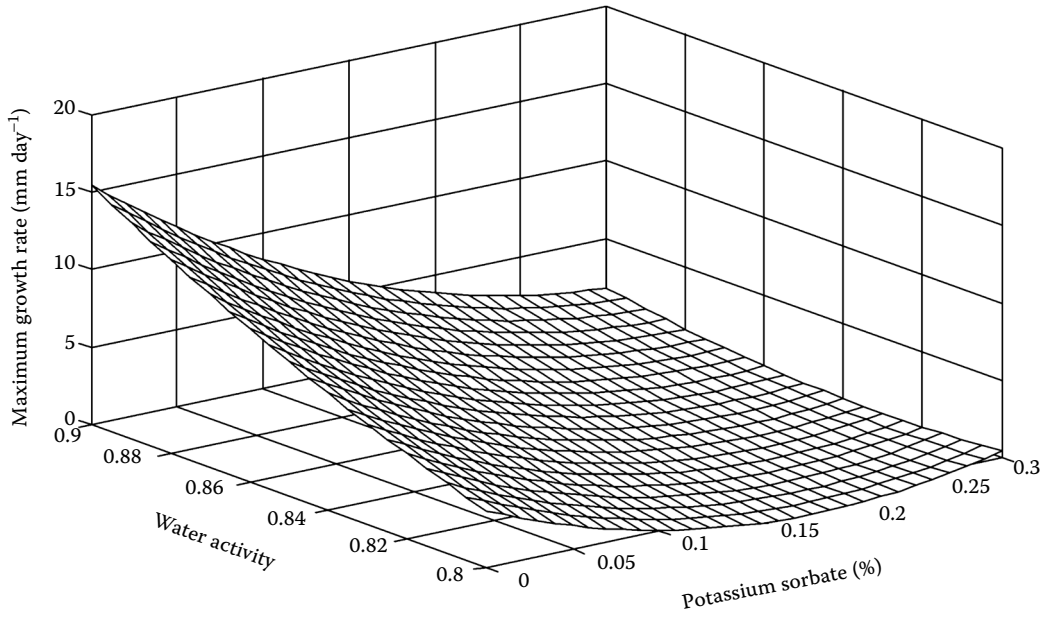

FIGURE 4.3 Maximum growth rate (μ_{max}) of a mold species, *Eurotium rubrum*, as a function of potassium sorbate concentration and water activity (a_w) at pH 5 and 25ºC. (Drawn from regression analysis from the data reported by Guynot M.E., Marıin S., Sanchis V., Ramos A.J. 2005. An attempt to optimize potassium sorbate use to preserve low pH (4.5–5.5) intermediate moisture bakery products by modelling *Eurotium* spp., *Aspergillus* spp. and *Penicillium corylophilum* growth. *International Journal of Food Microbiology* 101: 169–177.)

the optimal temperature, microbial enzymes required for their growth start to be denatured or inactivated. Below the optimum, enzyme activity in the microbial system, which is proportional to temperature, is reduced to decrease the microbial growth rate (Figure 4.4). Because packaged foods are usually distributed at or below ambient temperatures, microbial inactivation at high temperatures is not taken into consideration, and thus positive dependence of microbial growth or spoilage on temperature is usually assumed in shelf life determination. Microbial activity or growth is reduced at lower temperatures and stops below certain limit temperatures, which are given for some organisms in Table 4.1.

The Arrhenius equation is widely used for describing the temperature dependence of chemical reactions and is often adopted to represent the effect of temperature on microbial growth, which is given by the inverse of the lag time or the maximum specific growth rate (growth rate):

$$\frac{1}{t_{lag}} \text{ or } \mu_{max} = A \exp\left(\frac{-E_a}{RT}\right) \tag{4.4}$$

where A is a constant (the so-called frequency factor), R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature (K), and E_a is the activation energy (J mol^{-1}). E_a values for microbial growth usually range from 50 to 90 kJ mol^{-1}.

The square root (or Bêlehrádek) equation is also widely used to describe the temperature dependence of lag time or growth rate:

$$\sqrt{1/t_{lag}} \text{ or } \sqrt{\mu_{max}} = b\left(T - T_{min}\right) \tag{4.5}$$

where b is the slope parameter of temperature dependence ($hr^{-1/2}$ ºC^{-1} or $hr^{-1/2}$ K^{-1}) and T_{min} is a hypothetical minimum temperature extrapolated to zero growth (ºC or K) (see Figure 4.4).

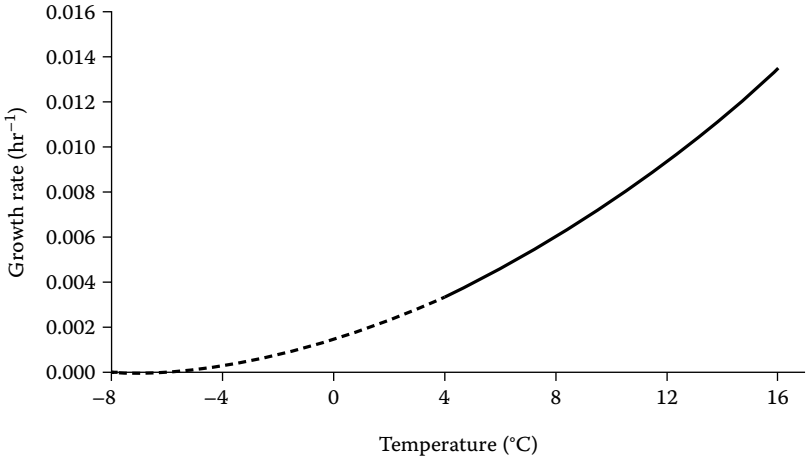

FIGURE 4.4 Temperature dependent growth rate (μ_{max}) of Pseudomonads with b of 0.00484 and T_{min} of –8.0°C (Equation 4.5) with dotted line part of extrapolation. E_a for Equation 4.4 is given as 77.2 kJ mol^{-1}. Based on the analysis of Vankerschaver K., Willocx F., Smout C., Hendricks M., Tobback P. 1996. The influence of temperature and gas mixtures on the growth of the intrinsic microorganisms on cut endive: predictive versus actual growth. *Food Microbiology* 13: 427–440.

Equation 4.5 is assumed to apply to temperature ranges from the minimum temperature to the optimal one for growth, and the T_{min} value is usually 2–3°C lower than the lower limit of growth (McMeekin et al., 1993). The T_{min} value ranges from –10°C to 7°C for most spoilage bacteria, such as Pseudomonads. However, it needs to be emphasized that chill or even freezing temperatures do not destroy or inactivate microorganisms, and storage temperature abuse after refrigeration or freezing can lead to the onset or resumption of microbial spoilage.

Package atmosphere is the second most important factor affecting the microbial growth rate. Carbon dioxide is widely used in MAP because it inhibits a wide range of spoilage and pathogenic microorganisms, particularly gram-negative bacteria and molds. Figure 4.5 shows the effect of CO_2 concentration on the growth of some spoilage bacteria. The suppression of microbial growth by CO_2 has been applied in MAP of perishable foods to extend their shelf life. However, the effectiveness of CO_2 in microbial growth suppression increases under chilled conditions, as the solubility of CO_2 in food is higher at lower temperatures (Lee et al., 2008a).

Other package atmosphere modifications also affect the rate of microbial spoilage: low O_2 concentrations below 0.21 atm slow down the growth of aerobic bacteria, yeasts, and molds. The microbial growth of aerobic microorganisms depends on dissolved O_2 in the media (corresponding to 0–0.21 atm of O_2 partial pressure in the air) and generally follows the empirical Monod equation for microbial growth (Figure 4.6A). The O_2 partial pressure of the package atmosphere, controlling the dissolved O_2 concentration, presumably affects microbial growth in a similar way; however, published studies on the kinetics of food spoilage organism growth as a function of package O_2 concentration are rare, except for simple qualitative reports on microbial spoilage (Hu et al., 2007). High O_2 concentrations (far above the normal atmospheric level) have recently been reported to inhibit some microorganisms (Figure 4.6B), and this approach has begun to be utilized for fresh produce packaging, which normally risks the creation of anoxic conditions due to respiration. The effect of superatmospheric O_2 on extending the lag time and reducing the growth rate for bacteria and yeasts has been reported to be greater in the presence of CO_2, with benefits for fresh produce packaging (Amanatidou et al., 1999; Conesa et al., 2007). It should be noted that microbial growth or activity in a hermetic package may modify the headspace gas composition by consuming O_2 and producing CO_2, which can in turn be utilized for slowing down the growth of microbes such as gram-negative Pseudomonads (Koutsoumanis et al., 2008).

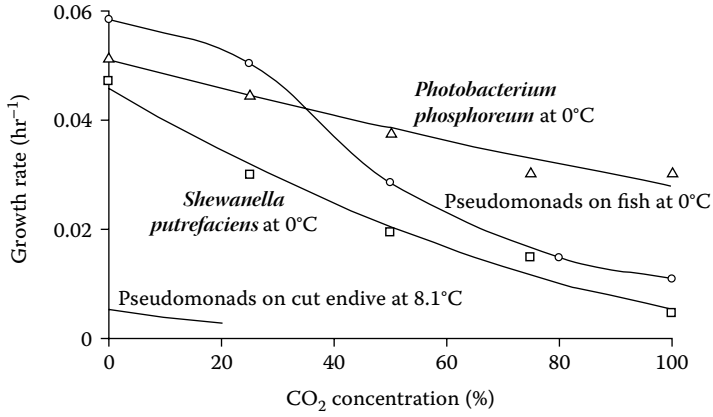

FIGURE 4.5 Effect of package CO_2 concentration on the growth rate (μ_{max}) of some SSOs. Based on experimental data and analyses of Pseudomonads on red mullet fish at 0ºC (Koutsoumanis et al., 2000), Pseudomonads on cut endive at 8.1ºC (Vankerschaver et al., 1996) and *Sh. putrefaciens* and *Ph. phosphoreum* in cooked fish muscle juice and broth medium, respectively, at 0ºC (Dalgaard, 1995).

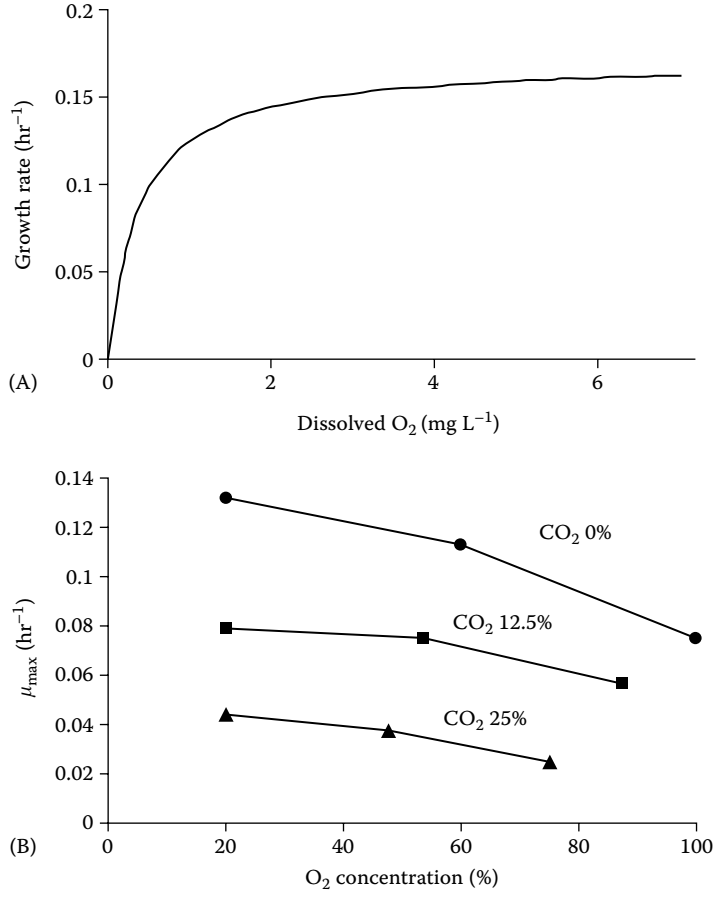

FIGURE 4.6 Effect of oxygen on aerobic bacterial growth. (A) Specific growth rate of a *Pseudomonas* sp. at 30ºC as a function of dissolved oxygen concentration (lower range corresponding to 0–0.21 atm of O_2 partial pressure) according to the kinetic information given by Ferreira Jorge and Livingston (1999). (B) Maximum growth rate (μ_{max}) of *Pseudomonas fluorescens* depending on superatmospheric oxygen concentration as reported by Geysen et al. (2005).

Other volatiles produced by fresh produce or deliberately delivered in small amounts into the package headspace by active packaging can inhibit microbial growth and thus extend the shelf life. Ethanol, hexanal, allyl isothiocyanate, and jasmonates are typical volatiles used to reduce fungal decay during the storage of fruits, vegetables, and cakes. Although some of these compounds are produced by senescent metabolic activity of fresh produce or degradation of other foods and may sometimes be undesirable, their selective removal and addition may be helpful for the preservation of perishable foods (Toivonen, 1997). Because active packaging is dealt with in Chapter 20, only a brief mention is made here.

Although the deteriorative effect of light (related to package transparency) on the chemical quality of packaged foods has been studied extensively (Robertson, 2006), its effect on microbial quality is rarely reported in the literature, and package transparency does not appear to have a significant influence on microbial activity. As a specialized application, TiO_2-coated films activated by light have been reported to inhibit microorganisms (Chawengkijwanich and Hayata, 2008). The mechanism is presumed to be oxidation of the polyunsaturated phospholipid component of the microbial cell membranes by hydroxyl radicals and reactive oxygen species generated by illumination of the TiO_2 surface.

4.4 FOOD FACTORS AND MICROBIAL ECOLOGY INFLUENCED BY PACKAGING

Intrinsic food factors are changed by the interaction of the packaged food with its packaging and the external environment. The headspace may work as a buffer between the food and the packaging material. Through all these interactions, moisture content (i.e., water activity), dissolved O_2 and CO_2 contents, and preservative concentration may be changed to affect the microbial flora and growth rate. Figure 4.7 shows a schematic of food–package–environment interactions in relation to microbial growth. In combination with other environmental factors such as temperature, the resulting domain may be located within the growth/no growth boundary or outside it; the possibility for some spoilage and pathogenic organisms to grow in certain foods may be excluded or neglected depending on the domain of intrinsic food factors and temperature. The food factors and temperature will also determine the relative growth rates of microbial species able to spoil the food. In the case of foods exposed to dynamic temperatures, thermal insulation may help to reduce the impact of the temperature variations.

When a packaged food is set up under initial conditions of preparation, microbial contamination, moisture, and package headspace composition, with a specific packaging material and stored at a specific temperature, permeation of water vapor, O_2, and CO_2 determines their respective concentrations inside the package, which in turn determines the moisture, O_2, and CO_2 sorbed or dissolved into the food (Figure 4.7). Usually it can be assumed that the transmission rates of these vapors or gases control the variation of these intrinsic food factors and equilibrium is maintained between the food and the headspace. The high solubility of CO_2 gas in wet or fatty foods may significantly change its initial concentration in the headspace after packaging, particularly when the headspace volume is not large. Whereas the outside gas concentrations of O_2 and CO_2 are taken as constant, humidity in the environment can vary with time. If the packaged food does not make any contribution to O_2 and CO_2 changes in the system, a gas barrier package can be used to maintain constant levels of O_2 and CO_2 in the headspace and thus in the food. High microbial activity, fresh produce respiration, and O_2 absorption by active packaging components may work as other variables affecting microbial growth, as may elaborate migration of preservative or vaporization of volatiles into the headspace.

Together with the intrinsic properties of the food, the storage temperature of the package determines the microbial population outgrowth: ambient storage favors mesophiles, and chilled storage results in the dominance of psychrotrophic organisms. The relative growth rates of microbial species, resulting from the different package conditions, determine the dominant microbial flora on

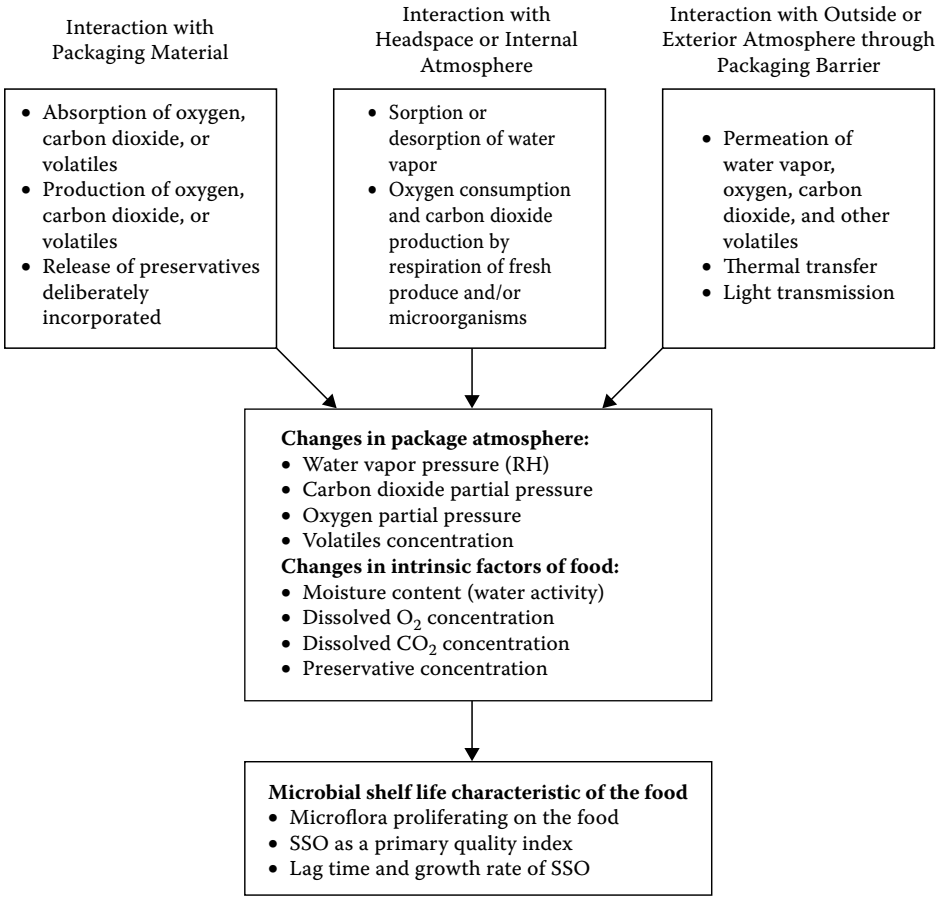

FIGURE 4.7 Food–package–environment interactions affecting the microbial shelf life characteristics of packaged foods.

TABLE 4.2
Influence of Package Atmosphere on the Microbial Group Counts of Pork Meat Stored at 4°C

Package Atmosphere and Storage	Aerobic Bacteria (log cfu cm^{-2})	Lactic Acid Bacteria (log cfu cm^{-2})	Coliforms (log cfu cm^{-2})
Initial	3.3	0.3	0.8
Air for 7 days	8.0	1.4	4.4
N_2 for 7 days	5.9	2.3	3.2
CO_2 for 7 days	3.6	2.4	0

Source: Adapted from Enfores S.-O., Molin G., Ternstrom A. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. *Journal of Applied Bacteriology* 47: 197–208.

the food. As shown in Tables 4.2 and 4.3, low O_2 or anoxic packages generally favor the growth of anaerobes or microaerophiles on the packaged foods and high CO_2 concentrations favor gram-positive bacteria (such as lactic acid bacteria) over gram-negative ones (such as *Pseudomonas* spp. and coliforms) (Banks et al., 1980; Enfores et al., 1979). Although it strongly inhibits the growth of gram-negative bacteria and molds, the inclusion of CO_2 in the package can also inhibit or delay, to

some extent, the growth of certain gram-positive bacteria and yeasts, and thus the microbial flora will differ from that in air (Table 4.3).

Temperature significantly affects the effectiveness of microbial inhibition of modified atmospheres (MAs) (Table 4.4). Compared with the anaerobic conditions of vacuum or N_2, a CO_2 atmosphere is the most effective at low temperatures. As mentioned, intrinsic factors such as pH and a_w also work to confine or decide the microbial spoilage types for perishable foods. Microbial

TABLE 4.3
Influence of Package Atmosphere on the Microbial Flora of Pork Meat Stored at 4°C

Organisms	Percentage of Organisms At			
	Initial State	7 Days in Air	10 Days in N_2	7 Days in CO_2
Acinetobacter calcoaceticus	49			30
Aeromonas hydrophila	3		5	
Bacillus subtilis		3		
Enterobacter liquefaciens	3			
Flavobacterium sp.	8			
Flexibacter sp.	3			
Kurthia zopfii	2		10	5
Lactobacillus plantarum			5	20
Micrococcus varians	2			
Moraxella sp.	5			
Pediococcus pentosaceus	1			
Pseudomonas spp.	24	97	80	45
Staphylococcus xylosus	1			
Aerobic bacterial count (log cfu cm^{-2})	3.3	8.0	7.3	3.6

Source: Adapted from Enfores S.-O., Molin G., Ternstrom A. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. *Journal of Applied Bacteriology* 47: 197–208.

TABLE 4.4
Effectiveness of Anaerobic Packaging Conditions Compared to Air Packaging at Different Temperatures

Package Atmosphere	Growth of Microorganisms as Percentage of Growth in Air after 23 Days at Temperature (°C):		
	2	6	20
Pseudomonas/Acinetobacter/Moraxella			
Vacuum	95	78	33
N_2	82	69	76
CO_2	0	0	52
Brochothrix thermosphacta			
Vacuum	60	69	63
N_2	45	57	63
CO_2	0	0	46

Source: Adapted from Eklund T., Jarmund T. 1983. Microculture model studies on the effect of various gas atmospheres on microbial growth at different temperatures. *Journal of Applied Bacteriology* 55: 119–125.

ecology resulting from storage temperature, package atmosphere, and intrinsic factors determines the primary microbial index to be used for shelf life determination. For example, counts of lactic acid bacteria have often been used as quality criteria for shelf life determination of chill-stored cooked meats and fresh vegetables packaged in vacuum, low O_2, or high CO_2 MAs, and counts of mesophilic or psychrotrophic aerobic bacteria have been used for aerobic packages of fresh meat, fish, and vegetables. Products with low a_w and pH, such as processed meats and pasta, in air or O_2-permeable packages are spoiled by growth of molds and yeasts. More specifically, *Pseudomonas* spp. were identified as SSOs for minimally processed vegetable products, milk, meat, and fish packaged under O_2-containing atmospheres, with their counts used to determine shelf life; *Ph. phosphoreum* has been used for MA-packed fresh fish; *Lactobacillus sake*, for high-CO_2-packed meat products; *Sh. putrefaciens* or *Br. thermosphacta*, for CO_2-packed fish and meat; and *Leuconostoc* spp., for high O_2 MA-packaged beef steak (Berruga et al., 2005; Devlieghere et al., 1999; Koutsoumanis et al., 2000; McMeekin and Ross, 1996; Sheridan et al., 1997; Vihavainen and Bjorkroth, 2007). Again, it must be emphasized that SSOs and primary microbial quality indices are determined by a combination of intrinsic and extrinsic factors, including packaging conditions.

4.5 EFFECT OF PACKAGE GAS BARRIER ON MICROBIAL SHELF LIFE

For MA packages (vacuum or gas flushed) designed to suppress the growth of aerobic microorganisms and oxidative quality changes by excluding O_2, packaging materials with a poor gas barrier act to promote microbial growth of aerobes and facultative anaerobes (Kotzekidou and Bloukas, 1996; Newton and Rigg, 1979). Even microaerophiles such as *Lactobacillus* spp., which dominate in vacuum and CO_2 packaging of meat products, may have enhanced growth rates with higher O_2 transmission film or packaging (Tsigarida and Nychas, 2006). The effect of gas permeability on microbial spoilage is seen clearly in Figure 4.8, in which a *sous vide* package with a high oxygen transmission rate (OTR) favors the growth of aerobic and anaerobic bacteria. The high microbial load consisting of thermoduric *Bacillus* spp., facultative anaerobes that survived the pasteurization process, was presumed to have been responsible for the microbial spoilage (Gould, 1996; Kim

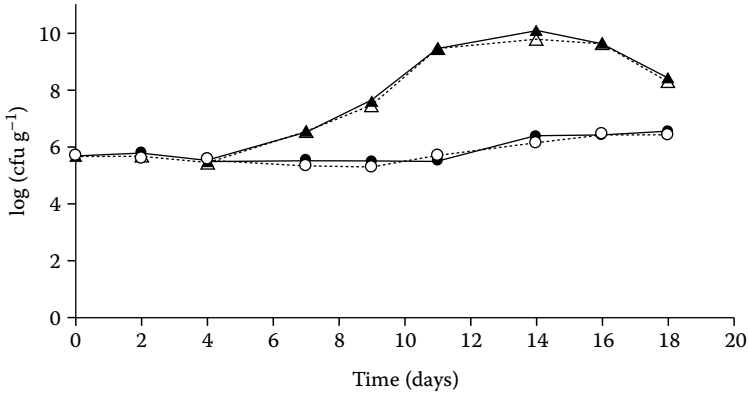

FIGURE 4.8 Effect of gas permeability on evolution of aerobic and anaerobic bacterial counts of *sous vide* packaged seasoned spinach soup (600-g pouch pack) at 10°C containing thermoduric organisms. ▲: aerobic bacteria with high-O_2-permeability film package (OTR 6.3 mL m^{-2} hr^{-1} assumed at 1 atm of O_2 partial pressure differential); Δ: anaerobic bacteria under high-O_2-permeability film package; ●: aerobic bacteria under low-O_2-permeability film package (OTR 2.3 mL m^{-2} hr^{-1}); ○: anaerobic bacteria under low-O_2-permeability film package. (Adapted from Kim G.T., Paik H.D., Lee D.S. 2003. Effect of different oxygen permeability packaging films on the quality of *sous vide* processed seasoned spinach soup. *Food Science and Biotechnology* 12: 312–315.)

et al., 2003). When the microbial lag time was used to estimate shelf life in Figure 4.8, a gas barrier film package with three times less O_2 permeation extended the shelf life to twice that for the more permeable one. In a comparison of an O_2-permeable package in air with a vacuum package with a high gas barrier (two extremes in O_2 transmission), the latter could have an extended shelf life, particularly at lower temperatures, as shown in Figure 4.9.

Figure 4.10 presents shelf life estimates of vacuum-packed meat based on different microbial criteria. Shelf life based on *Pseudomonas* growth (which correlated strongly with sensory odor) was a strong function of the gas permeability of the packaging film, whereas growth of lactic acid

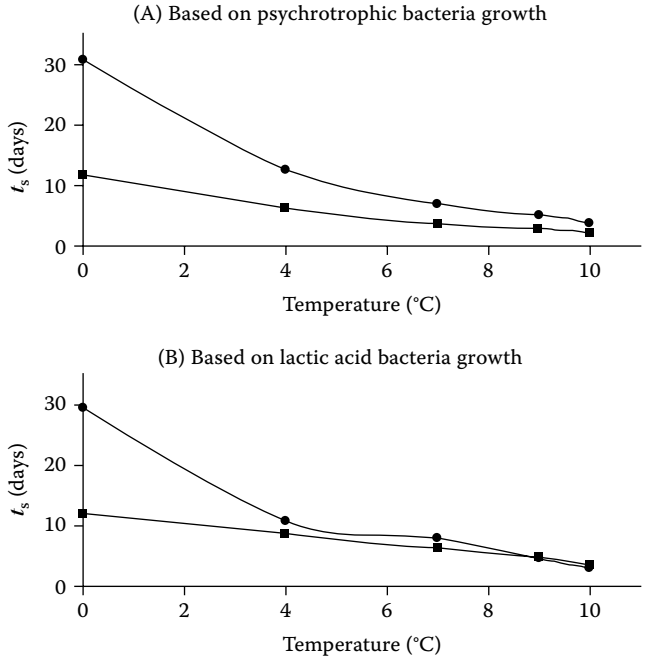

FIGURE 4.9 Microbial shelf life (t_s) of chilled packaged beef estimated from microbial growth model parameters reported by Giannuzzi et al. (1998). The t_s was obtained according to Equation 4.3 as the time for psychrotrophic and lactic acid bacteria to increase by 10^3- and 10^2-fold, respectively. ●: high-barrier poly(vinylidene chloride) (PVdC) vacuum package; ■: gas-permeable low density polyethylene air package.

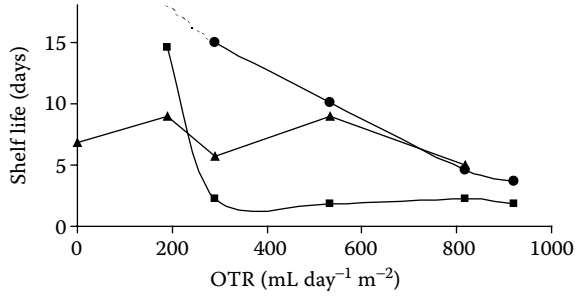

FIGURE 4.10 Different criteria shelf lives of vacuum-packed meat at 0°C as a function of OTR at 25°C and 100% RH. ●: time to reach 5×10^5 organisms g^{-1} of *Pseudomonas*; ▲: time to reach 10^6 organisms g^{-1} of *Lactobacillus*; ■: time to reach 10^5 organisms g^{-1} of *Brochothrix thermosphacta*. Values were read from graphical data of Newton and Rigg (1979), with dotted line being an extrapolated value.

bacteria to 10^6 organisms g^{-1} depended little on the gas permeability. Growth of lactic acid bacteria on vacuum-packed cooked meat was also shown by others to be little affected by the OTR of the packaging film (Cayre et al., 2005; Kotzekidou and Bloukas, 1996). *Br. thermosphacta* growth could be suppressed significantly only with very high gas barrier film (Figure 4.10). The behavior of *Br. thermosphacta* with different gas barrier packages was similar to that reported by Kotzekidou and Bloukas (1996) for vacuum-packed cooked ham, but not to that by Cayre et al. (2005), who reported a dramatic decrease in *Br. thermosphacta* count after earlier maximum with high OTR film for vacuum-packed cooked meat emulsions. A difference in intrinsic factors and microbial interactions may have resulted in different microbial spoilage behaviors even under similar packaging conditions.

In the case of CO_2 packaging of a meat product, high gas barrier packaging was found to be effective in extending the shelf life consistently over the chilled temperature range (Figure 4.11). A comparison between Figures 4.9 and 4.11 shows the temperature dependence of the gas barrier effect to be more pronounced with vacuum packaging than with CO_2 packaging. Some accumulation of CO_2 from microbial activity of the product in vacuum packages may explain the greater effect of barrier packaging at lower temperatures, due to the higher antimicrobial activity of CO_2 at lower temperatures, as explained earlier. Koutsoumanis et al. (2008) also observed a greater effect of high gas barrier film on extending the shelf life of minced pork at lower temperatures that resulted from an MA arising from microbial respiration. Although gas barrier properties are an important variable to reduce the microbial growth rate for vacuum or gas-flushed packages, there seems to be a threshold value of OTR below which microbial growth or shelf life is independent of these. Tsigarida and Nychas (2006) showed that shelf life was not extended further below an OTR of 28 mL day^{-1} m^{-2} based on a 1-atm partial pressure difference (23°C and 75% RH). Generally speaking, the packaging material should provide enough of a barrier to protect against O_2 ingress or CO_2 loss, or both, to have the desired effect from vacuum or gas-flushing.

Respiring fresh produce can retain better microbial quality when the design of the package is such that it can attain the optimal equilibrated atmosphere. This requires a selective range of permeabilities to O_2 and CO_2. As long as the physiological tolerance limits for O_2 and CO_2 concentrations are not violated, higher CO_2 and lower O_2 (below 21%) help ensure lower counts of spoilage organisms such as *Pseudomonas* (Charles et al., 2005; Simon et al., 2005; Vankerschaver et al., 1996).

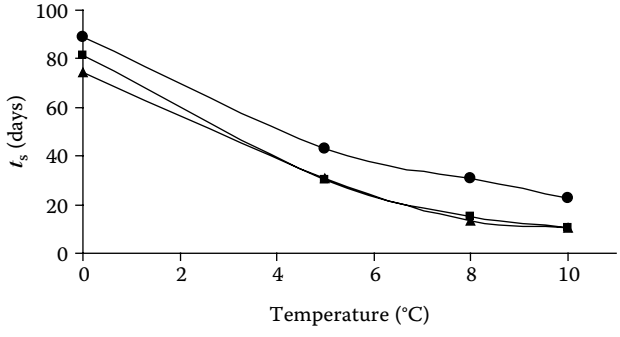

FIGURE 4.11 Shelf life (t_s) of 100% CO_2-packed meat fillets at different temperatures versus OTR of packaging film at 23°C and 75% RH. The t_s was calculated according to Equation 4.3 as the time for *Lactobacillus* spp. to increase by 10^3-fold by using the microbial growth kinetic parameters given by Tsigarida and Nychas (2006). ●: OTR 28 mL day^{-1} m^{-2}; ■: OTR 2600 mL day^{-1} m^{-2}; ▲: air pack.

4.6 MODIFIED ATMOSPHERE PACKAGING TO EXTEND MICROBIAL SHELF LIFE

MAP is an effective technique to preserve perishable chilled foods without resorting to heat processing or chemical preservatives. As discussed earlier, the preserving effect of MAP derives mainly from the use of CO_2 gas, which inhibits or retards microbial growth significantly above a concentration of 20%. Sometimes a lower O_2 concentration is used alone in packaging with a vacuum or in combination with a high CO_2 concentration. Low O_2 and moderate accumulation of CO_2 provide some degree of microbial inhibition in the permeable packaging of fresh produce with a reduction of physiological senescence. Recently, high O_2 concentrations have been applied mainly for fresh-produce packaging to inhibit microbial growth without the creation of anoxic conditions. As many studies have already reported the beneficial effects of MAP, this chapter gives an overview of the effect of MAP conditions on the extension of microbial shelf life by compiling specific data for a variety of foods.

The dependence of microbial shelf life on superatmospheric O_2 and moderate CO_2 concentrations for fresh produce is presented in Figure 4.12, which estimates shelf life according to Equation 4.3 using growth kinetic parameters from the literature (Geysen et al., 2006). Superatmospheric O_2 concentrations are more effective with high CO_2 concentrations in extending microbial shelf life: the maximum extended shelf life at 100% O_2 and 20% CO_2 (hypothetical concentrations corresponding to respective partial pressures of 1.0 and 0.2 atm for O_2 and CO_2) is about six times that in a normal atmosphere (20% O_2 and 0% CO_2). Use of higher levels of CO_2 for fresh fruits and vegetables is limited because of the physiological tolerance limit of the commodity (mostly below 20%). Given the microbial inhibitory effect of superatmospheric O_2, its use eliminates the risk of O_2 depletion inside the fresh produce package because of respiration activity, particularly under temperature-abuse conditions.

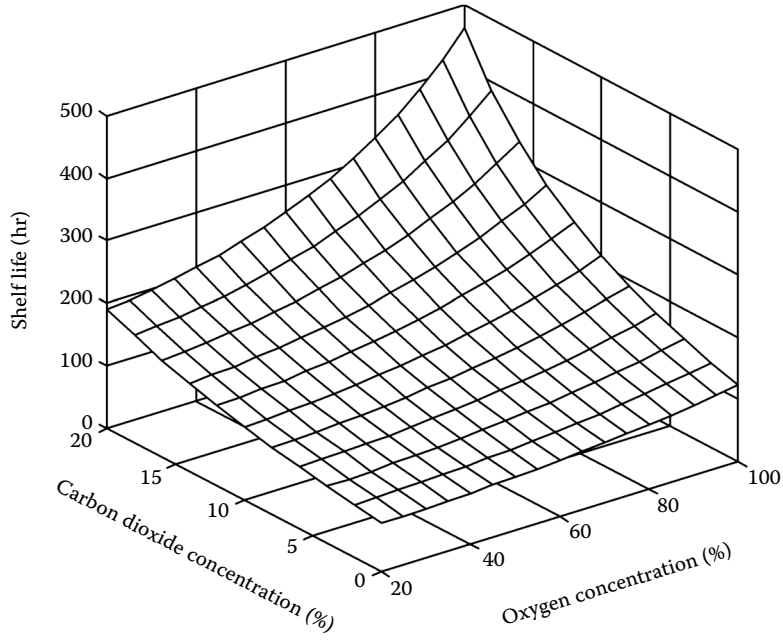

FIGURE 4.12 Estimated shelf life under MA conditions of superatmospheric O_2 and moderate CO_2 concentrations when packaging fresh-cut lettuce at 7°C. Equation 4.3 was used to obtain the time for *Pseudomonas fluorescens* to increase $10^{3.5}$-fold by using the microbial growth model parameters given by Geysen et al. (2006).

Although some level of O_2 may be used to bloom or retain the bright red color of fresh meat, the effectiveness of microbial inhibition for flesh and nonrespiring food products is mostly due to CO_2, the effect of which is more pronounced at lower chill temperatures. Figure 4.13 shows the effect of CO_2 concentration on the extension of microbial shelf life on the basis of the growth of Pseudomonads on fish. The effectiveness of shelf life extension using 100% CO_2 (compared with 0% CO_2) at 0°C was found to be about 480% (1130 vs. 196 hr). This effectiveness of CO_2 on fish Pseudomonads is much higher than the time period estimates for *La. sake* to go from 5×10^2 to 10^7 cells mL^{-1} in modified brain heart infusion medium at 4°C (about a 70% extension with dissolved CO_2 concentration of 2000 ppm when compared to 0 ppm CO_2) (Devlieghere et al., 1998). Gram-negative Pseudomonads would be more sensitive to CO_2 than gram-positive, microaerophilic *La. sake*.

Apart from the theoretical kinetic analysis given earlier, practical MAP applications for extending microbial shelf life use a variety of conditions for preserving the overall quality attributes of commodities. Table 4.5 is a compilation of MAP applications for extending the shelf life of perishable foods. The effectiveness of microbial shelf life extension varies with product and the actual MA applied. However, it is apparent that CO_2 inclusion is an important variable to give a significant shelf life extension for meat, fish, dairy, and prepared products. Bulk packaging or master packs of individual retail gas-permeable packs under a CO_2 atmosphere are often used for extending the total shelf life of flesh products from the wholesaler through retail display to consumers. A residual effect of CO_2 in inhibiting microbial spoilage after moving individual packs to air has sometimes been observed, presumably due to the microbial ecology formulated with MAP (Dixon and Kell, 1989). However, specific data on its effect on shelf life are not available in the published literature.

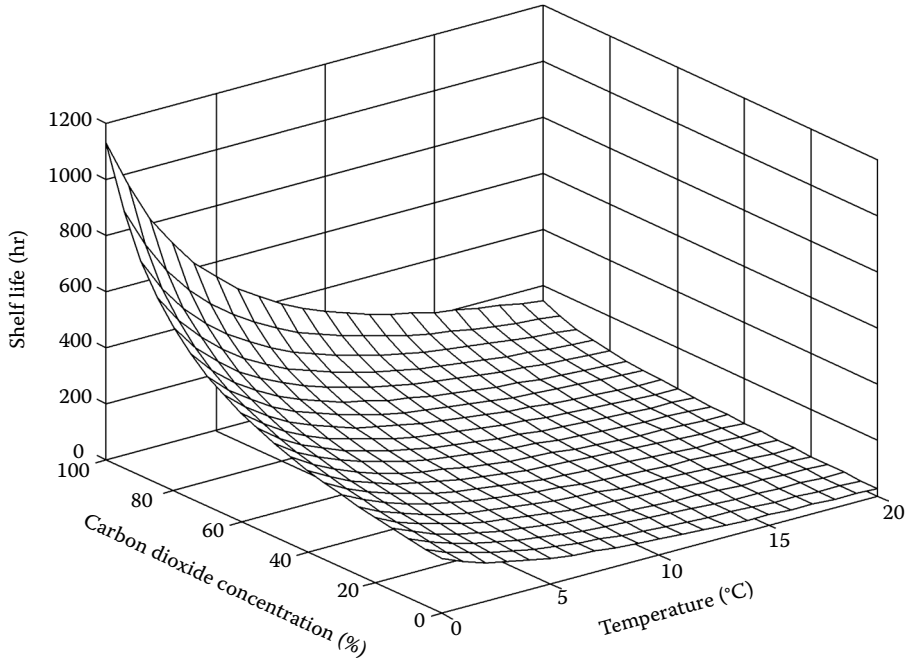

FIGURE 4.13 Shelf life estimates as a function of CO_2 concentration ($[CO_2]$) and temperature (T), based on the time for Pseudomonads to increase 10^4-fold. The maximum specific growth rate (μ_{max}) given in a Bêlehrádek-type equation $\left(\sqrt{\mu_{max}} = 0.00173(T + 11.4)\sqrt{120.9 - [CO_2]}\right)$ by Koutsoumanis et al. (2000) was used to calculate the shelf life by Equation 4.3 with absence of lag time.

TABLE 4.5
Compilation of Data on Microbial Shelf Life Extension Achieved by Active MAP of Food

Food	Package and Storage Conditions	Criterion for Microbial Quality Limit (cfu g^{-1} or cfu cm^{-2})	Increment of Shelf Life Extension Compared to Control (Air) Package	Reference
Meats				
Lamb meat	80% CO_2/20% N_2 at 2°C	10^7 of aerobic bacteria	140% compared to vacuum pack	Berruga et al. (2005)
Minced chicken meat in combination with irradiation	20% CO_2/80% N_2 at 10°C	10^7 of aerobic bacteria	80%	Grandison and Jennings (1993)
Ostrich steaks	30–80% CO_2/balance N_2 at 2°C	10^7 of aerobic bacteria	50%	Fernandez-Lo´pez et al. (2008)
Pork	70% CO_2/29.5% N_2/0.5% CO at 0–2°C	10^6 of aerobic bacteria	410%	Krause et al., (2003)
Pork sausage	99.6% CO_2/0.4% CO at 4°C	10^7 of aerobic bacteria	100%	Laury and Sebranek (2007)
Smoked turkey breast fillets	30% CO_2/70% N_2 at 4°C	10^7 of aerobic bacteria	30%	Ntzimani et al. (2008)
Fish				
Freshwater crayfish	80% CO_2/20% air at 4°C	10^7 of aerobic bacteria	>100%	Wang and Brown (1983)
Gutted bass	50% CO_2/30% O_2/20% N_2 at 3°C	10^4-fold increase in aerobic bacterial count	>>30%	Torrieri et al. (2006)
Pearlspot fish	60% CO_2/40% N_2 at 0–2°C	10^7 of psychrotrophic bacteria	>120%	Ravi Sankar et al. (2008)
Rock fish fillets	80% CO_2/20% air at 1.7°C	10^7 of aerobic bacteria	>200%	Parkin et al. (1981)
Shrimp	100% CO_2 at 4°C	10^7 of aerobic bacteria	>200%	Lannelongue et al. (1982)
Dairy products				
Cottage cheese	100% CO_2 at 8°C	10^2 of coliform bacteria or 10^3 of yeasts/molds	160%	Mannheim and Soffer (1996)
Sliced Mozzarella cheese	100% CO_2 at 7°C	10^7 of yeasts	>420%	Alves et al. (1996)
Whey cheese	40% CO_2/60% N_2 at 4°C	10^7 of mesophilic bacteria	310% compared to vacuum or air pack	Dermiki et al. (2008)
Fresh fruits and vegetables				
Endive	3% O_2/5% CO_2 at 20°C	10^6 of *Pseudomonas* spp.	>50%	Charles et al. (2005)
Fresh-cut lettuce	100% N_2 at 1, 5, and 10°C	10^7 of total aerobic bacteria	Negligible	Koseki and Itoh (2002)
Fresh-cut pineapple	40% O_2/balance N_2 at 5°C	10^7 of mesophilic bacteria	>30%	Montero-Calderon et al. (2008)
Fresh-cut baby spinach	100% O_2 at 5°C	10^8 of mesophilic bacteria	220%	Allende et al. (2004)
Fresh-cut spinach	0.8% O_2/10% CO_2 at 5°C	10^8 of mesophilic bacteria	80%	Babic and Watada (1996)
Shredded chicory endive	95% O_2/5% N_2 at 4°C	10^5 of yeasts	100% compared to passive MAP	Jacxsens et al. (2001)

(*Continued*)

TABLE 4.5 (*Continued*)

Food	Package and Storage Conditions	Criterion for Microbial Quality Limit (cfu g^{-1} or cfu cm^{-2})	Increment of Shelf Life Extension Compared to Control (Air) Package	Reference
Prepared or miscellaneous foods				
Carrot juice	100% CO_2 at 17°C	10^6 of aerobic bacteria	109%	Alklint et al. (2004)
Fermented, seasoned soused roe of Alaska pollack	30% CO_2/70% N_2 at 10°C	10^7 of yeasts	>500%	Lim et al. (2002)
Fresh wet pasta	22% CO_2/78% N_2 at 8°C	10^6 of aerobic bacteria	>150%	Lee et al. (2001)
Korean braised green peppers with dry anchovies	60% CO_2/40% N_2 at 10°C	10^5 of total aerobic bacteria	130%	Lee et al. (2008b)
Korean braised kidney beans	60% CO_2/40% N_2 at 10°C	Lag time of yeast/mold growth	500%	Lee et al. (2009)

For meat packaging, inclusion of small amounts of carbon monoxide (CO) has been found to give a further extension of microbial shelf life, with the added benefit of red color stabilization (Laury and Sebranek, 2007).

The microbial stability of fresh produce can benefit greatly from a combination of superatmospheric O_2 and self-produced CO_2 (Table 4.5). Although this approach has recently received considerable interest from researchers, it has not yet been adopted by industry. Today, fresh produce packaging depends on low O_2 and slightly increased CO_2 concentrations to prolong freshness by reducing respiration and softening. Some volatile compounds, such as methyl jasmonate, have been reported to decrease the fungal decay of minimally processed fruits in the package, but no specific data on shelf life extension can be found in the literature. Even though nonconventional gases such as Ar, Xe, and N_2O have also been applied for fresh-cut fruits to minimize physiological changes, their effect on microbial quality has not been reported. More work is needed to examine these aspects of microbial inhibition and physiological preservation.

4.7 PACKAGING TOOLS TO MONITOR MICROBIAL SHELF LIFE

Because of its importance in shelf life control and its high dependence on food distribution conditions, real-time monitoring of microbial quality of packaged food in the food supply chain has been desired and tried for a long time. The destructive measurement of microbial counts is very time consuming and requires laborious laboratory testing, and thus is not feasible for practical application in food logistics. Therefore, there have been attempts to use sensor technology to monitor microbial quality, detect spoilage, or predict shelf life under dynamic distribution environments.

A simple but reasonable approach is to predict the microbial quality change on the basis of the temperature history experienced in food supply chain. The quality change in response to temperature fluctuations can be expressed or shown as a color change in a time-temperature indicator (TTI) or the remaining shelf life predicted using a digital device. This approach requires shelf life prediction models for the particular food as a function of environmental conditions. Currently, temperature is the only variable that has been successfully taken into consideration. A TTI can be attached as a label on the package surface to respond to the external temperature, indicating the microbial quality change whose kinetics parallel the indicator color change. Taoukis et al. (1999) developed an algorithm for controlling the stock rotation and shelf life of chilled fish to have better

quality delivered to consumers by using a TTI that responded in the same way as microbial deterioration. Smolander et al. (2004) observed a high correlation between microbial quality of chicken cuts and TTI color change, which can be a useful tool to estimate the shelf life in real time. There is a need for a variety of TTIs to represent the microbial quality change due to SSO growth for many different foods. Common TTIs available commercially include Fresh Check™ and Vitsab™. Other temperature-sensing data loggers or devices can also be used for similar purposes. Recently, a radio frequency identification (RFID) tag incorporating a temperature sensor with data communication and calculation functions has been proposed and is being developed to support information management in the food supply chain. For a TTI or RFID tag system to be applied widely for shelf life detection in the food supply chain, more quantitative kinetic data and models for microbial food spoilage need to be accumulated.

Microbial food spoilage accompanies changes in the concentration of metabolic substrates and products, producing discoloration, textural changes, slime formation, and off-flavor development. Attempts to measure or monitor microbial food quality more directly use substrates or products of microbial growth or spoilage as the index. Typical indices for microbial spoilage are glucose, organic acids such as gluconic acid and lactic acid, ethanol, biogenic amines, volatile nitrogen compounds, adenosine triphosphate (ATP) degradation products, several alcohols, and H_2S (Dainty, 1996; Ellis and Goodacre, 2001). Generally, carbohydrates are degraded before amino acids and lactic acid are metabolized by microorganisms to impair the sensory quality of proteinaceous foods. The label or tag attached inside a transparent package surface is equipped with a sensor to measure one of these compounds closely related to food spoilage. The sensor label or tag is designed to cause a color change by reaction with one of these metabolites in the spoiled food. Among the metabolites, measurement of the volatile compounds accumulated in the package headspace is more suited for shelf life control of packaged perishable foods, because this does not require direct contact between the food and the sensor. Some prototype products warning of microbial spoilage are available commercially. Recently an electronic nose sensor has been tried for this purpose.

Regarding volatile compounds as microbial spoilage indicators, CO_2 and 3-methyl butanol have been found to be highly correlated with growth of *Br. thermosphacta* (Sutherland, 2003). Guerzoni et al. (1990) showed a high correlation between CO_2 production and *Saccharomyces cerevisiae* growth, which leads to the spoilage of peach products. According to Haugen et al. (2006), detection of CO_2, acetoin, acetate, or ethanol coincided with the start of the exponential growth of spoilage organisms inoculated into a model milk food. Analysis of volatiles in bakery products using an electronic nose also had the potential to detect and differentiate between spoilage by bacteria, yeasts, or fungi (Needham et al., 2005). Development of relevant sensors with adequate sensitivity to metabolites characteristic of the spoilage organism is required and is expected to be combined with food packaging and logistic tools in the near future, which can be realized by intelligent packaging systems adopting an information transfer function (Yam et al., 2005).

4.8 CONCLUSIONS AND PROSPECTS

Microbial growth or deterioration is considered the most important quality criterion for shelf life determination of most perishable food products because of its high relationship to food spoilage and safety. With the proliferation of chilled foods in the market, there will be more attention to and interest in microbial quality preservation and shelf life control, because there are potential risks and the chance of quality loss due to their intrinsically perishable nature and mishandling during distribution such as temperature abuse. Several packaging technologies, including MAP and intelligent packaging, have been developed to enhance microbial quality stability and safety. These advanced technologies will contribute to the delivery of high-quality food with extended shelf life to consumers. However, there is still a paucity of available quantitative information on shelf life extension conferred by advanced packaging techniques. The effect of new packaging techniques on microbial

flora and SSO selection needs to be examined. Accumulation of data describing the dependence of microbial shelf life extension on packaging variables is required for different types of foods with different intrinsic properties. The current practice of trial and error in designing packages to give the desired shelf life would benefit from and be improved by the systematic accumulation and analysis of storage stability data for different packaging and storage conditions. With developments in predictive microbiology, extensive and advanced mathematical models incorporated into computer software that can handle different packaging materials and other variables for a wide range of spoilage organisms will facilitate the estimation of microbial growth and food shelf life.

The traditional approach of shelf life estimation as a fixed time period at a specified temperature, which ignores temperature variations through the food supply chain, is no longer adequate. Online monitoring and display of the remaining shelf life attracts great interest from consumers, retailers, and manufacturers, who are nowadays more concerned about the quality and safety of foods. Predicting or monitoring the growth of spoilage organisms on a real-time basis is required for controlling food shelf life on the basis of microbial food quality. Intelligent packaging devices such as TTIs and other sensors may serve this function effectively, but kinetic models of microbial growth and an understanding of the deterioration mechanisms are prerequisites.

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